Use of patient data to derive long term random error in laboratory assays: Application to glycohemoglobin testing

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Abstract

- ***Context and Background.*** The measurement of glycohemoglobin is the best measure of mean glucose within a three month range. As it is used for patient education, counseling, feedback control and ultimately for patient motivation, its measurement should be optimally accurate and precise. Estimates of imprecision are usually based on the repeated analysis of reference samples. These estimates are dependent on the reference sample's characteristics and where it enters the analytical stream.
- ***Objective.*** We describe a novel approach for deriving total imprecision of glycohemoglobin assays in which intra-individual glycohemoglobin variations are plotted against the time between sampling. Extrapolation to zero time will yield the total random error.
- ***Methods.*** Glycohemoglobin measurements of pairs of outpatient blood samples drawn between 0 and 30 days were made on the Bio-Rad Variant II's cation exchange high performance liquid chromatography (HPLC) assay (Hercules, CA), and the Beckman LX-20 turbidimetric immunoinhibition system (Beckman Coulter, Fullerton, CA). The average variation of grouped duplicates was calculated and graphed against corresponding time intervals. Regression to the y-intercept (0 day separation between readings) was used to determine the analytic variation.

Introduction

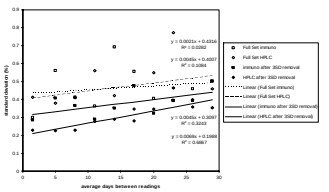
- Glycohemoglobin provides a three-four month estimate of mean glucose and is the best measure of long-term glucose control. It is used for patient education, counseling, feedback control, and ultimately, patient motivation and its measurement should be optimally accurate and precise.
- Based on hemoglobin A1c variation in intensively controlled type I diabetes patients, it has been proposed that long-term analytical coefficients of variation (CVs) be no more than 2.1%. This maximally allowable CV is lower than those recommended by the National Glycohemoglobin Standardization program of 3% for laboratories involved in clinical trials and 4% for reagent/instrument manufactures.
- In the summer of 2003, we discontinued the measurement of glycohemoglobin (hemoglobin A1c) by the Bio-Rad Variant II's cation exchange high performance liquid chromatography (HPLC) assay (Hercules, CA). Instead, we began to use the turbidimetric immunoinhibition assay provided by our Beckman LX-20 systems (Beckman Coulter, Fullerton, CA). Approximately 6 months later, because of physician complaints of excessive HbA1c variation, we reverted to the Bio-Rad Variant II.
- From this natural experiment, we were able to summarize and compare the total (physiological and analytical) variation in HbA1c values for the two systems in the same patient population. Plotting variation against time allowed determination of the analytical error component of the two systems.

Results

- The Table shows the number of paired HbA1c data obtained for each time interval.
- In the Figure, average SD of duplicate readings is plotted against time for each interval. The total variation of the immunoassay is relatively constant with most points, reflecting the immunoassay's analytical SD. The total variation of the HPLC increases with time.
- Analytical SDs as derived from the y-intercept are 0.31% and 0.20% for the immunoassay and HPLC, respectively.
- Analytical CVs were calculated by formula CV = 100 x (SD/population mean). Respective analytical CVs for the immunoassay and HPLC were 4.4% and 2.8%. The population means were 7.0% for both immunoassay and HPLC.
- The 99% confidence limits for an actual HbA1c of 7.0% measured on the immunoassay would be 6.2-7.8%, while the same confidence limits for the HPLC assay are a narrower 6.5-7.5%.

Figure

Average SD of HPLC (full and empty diamonds) and immunoassay (full and empty squares) versus time between testing. The y-intercept represents the average analytical SD, while graphs represent both analytical and physiological variation.



Abstract

- ***Results.*** 2707 and 774 pairs of HPLC and immunochemical glycohemoglobin values were obtained with the time between sampling varying from 0 to 30 days. After outlier removal, analytic coefficients of variation (CVs) for the HPLC and immunoassay were determined as 2.8% and 4.4%, respectively
- ***Conclusions.*** The immunochemical assay's random error, at 4.4%, significantly exceeds the maximum limits for random error established by biologic variation (2 to 3%) as well as the limits of the National Glycohemoglobin Standardization Program (3% for laboratories involved in clinical trials and 4% for reagent / instrument manufacturers and other laboratories). In contrast, the random error of the HPLC method, at 2.8%, appears to be acceptable. This approach to deriving total imprecision should be extended to all available glycohemoglobin analyzers.

Methods

- Continuing our previous work with HbA1c data, sequential patient readings were analyzed with a programmed query-based Visual Basic Microsoft Access file. A total of twenty-seven months of serial HbA1c data were collected within overlapping periods: between 2/3/2002 and 10/27/2004 using the Bio-Rad HPLC method and from 6/1/2003 to 11/16/2003 using the Beckman immunoassay method. We set the maximum time period for the collection of intrapatient duplicates to 30 days.
- From a total of 52,272 patient values (10953 from the immunoassay, 30139 from HPLC) there were 774 immunochemical HbA1c pairs and 2707 from the HPLC assay after outliers exceeding 3.0 SDs were removed. The Table shows the number of patients who had HbA1c ordered.
- Paired data were grouped into three-day time intervals (patient readings repeated between 0 and 3 days, 4 and 6 days, etc.). Average variation of these groups were calculated from the formula for the standard deviation of duplicate readings: s = v[(Σ (x1-x2)²/2)/n), and was then graphed against the midpoint of the time interval.
- Linear regression analysis allows extrapolation of the variation to time zero, where intrapatient HbA1c variation will be zero and the average variation will correspond to the average analytical variation over the period that the HbA1c pairs were collected (see Figure).

Table

The number of pairs corresponding to each three-day interval (of each data point).

Midpoint of time intervals, days	*HPLC n (full set)*	*HPLC n (after 3SD outlier removal)*	*immunoassay n (full set)*	*immunoassay n (after 3SD outlier removal)*
1.5	544	528	161	159
5	189	181	47	44
8	240	228	71	69
11	132	124	33	32
14	281	276	93	89
17	159	156	32	31
20	252	246	64	63
23	185	177	53	53
26	228	225	77	75
29	579	566	159	159
total	2789	2707	790	774

Conclusions

- We believe that a manufacturer's usually cited HbA1c variation reflects the variation in a single lot of reagents. This analysis considers the variation observed in specimens separated over periods of up to 30 days. A twenty seven month period with the HPLC system was overlapped with a five month immunoassay period. The patient duplicate variation in the Variant thus represents a measure of the average variation in a period exceeding one year. Moreover, the plotting of random error versus an extended time between duplicates reduces the potentially confounding effect of seasonality (HbA1c levels are more constant from August to December). Although individual time points could be plotted, time intervals provide a more visually informative graph.
- The Beckman immunochemical assay's random error, at 4.4%, significantly exceeds the maximum limits for random error established by biologic variation (2 to 3 %) as well as the limits of the National Glycohemoglobin Standardization Program (3% for laboratories involved in clinical trials and 4% for reagent / instrument manufacturers and other laboratories). In contrast, the random error of the Bio-Rad HPLC method, at 2.8%, appears to be acceptable. We recommend that this approach be used to quantitate the imprecision of all HbA1c systems.